ss(-)RNA Viruses

Introduction

The viruses in this lesson are all single-stranded negative-sense RNA viruses, abbreviated ss(–)RNA. The fact that you are learning them together in a single lesson will help carve out a spot in your memory, a mental cluster. This lesson (and Figure 5.1) serves as the advanced organizer to help you recall which viruses are ss(–)RNA, right down to the visualization of the viruses with the strikethrough marks. When you get a question about a virus, summon an image of the whiteboard in your mind, see where that virus is located on the board, and look around that memory of the board. The familiarity will help trigger the knowledge of which category the virus belongs to.

ss(–)RNA viruses share structural and replication similarities, as discussed in the next section. Unlike any other viruses, because they are negative-sense, they **must bring their own RNA-dependent RNA polymerase (RdRp)** with them to start an infection. The viral RdRp uses the antisense genome both to generate multiple mRNAs that direct host ribosomes to the synthesis of viral proteins and to generate an intact genome template for that same RdRp to use to transcribe negative-sense genome, to replicate the virus.

The replication template is accessed by RdRp to replicate more antisense viral genome. The mRNA is accessed by ribosomes to make protein.

We attempt to limit your learning to the viruses that matter to human disease in the United States. There are many ss(–)RNA viruses science knows a lot about. However, this lesson has the fewest viruses discussed yet, and we purposefully ignore several of the viruses that are negative-sense. We consciously do not discuss, only list, *Filoviridae* (Ebola), *Bunyaviridae* (Hanta), and *Arenavirus* (Lassa fever), because they are untreatable, have no vaccine, come from endemic areas outside the United States, and are usually fatal. They are sensational and make for great movies (and TV, as in the Ebola outbreak of 2014 where one person who already had the virus came to the States and died). Which means, on a more practical level, you should ignore those.

Figure 5.1: Negative-Sense RNA Virus Map
As we have done in the last two lessons, we work across this map left to right, increasing in virus size as we go. By far, the highest-yield organism is influenza, which is delivered last because it is the largest of the ones we discuss. You may have noticed those greyed-out virus families. We don't teach you those. If you are studying to practice medicine within the United States, there is no reason to learn them.

ss(–)RNA Virus Reminder

We learned from the first two lessons in this virus series that we can make generalizations about the structure and function of all the viruses listed in Figure 5.1 because they are all ss(–)RNA viruses, therefore limiting the amount of detail we'll need to memorize. Here it is again summarized.

All ss(–)RNA viruses are **single-stranded**.
All ss(–)RNA viruses are **helical**, and being helical necessitate an **envelope**. Being enveloped, these viruses tend to not be cytolytic, though they do cause severe acute illnesses.
All ss(–)RNA viruses replicate in the **cytoplasm**.
All ss(–)RNA viruses bring with them **RNA-dependent RNA polymerase**.

The only exception is influenza: it obeys all of these rules, except it replicates in the **nucleus**.

From here on, we go virus by virus. We aren't going to say, "single stranded, helical, must bring RdRp," in every virus. You can assume this section is applied to all the viruses that follow. No exceptions will be mentioned.

Paramyxovirus

Parainfluenza, respiratory syncytial virus (RSV), mumps, and measles are all of the *Paramyxoviridae* family. Parainfluenza and RSV affect the respiratory tract of children, mumps targets the testes and parotid gland, and measles causes a fever and a rash. There are many details about *Paramyxoviridae* genera, particularly in the attachment and fusion proteins that differentiate the viruses from one another. We're leaving those out in favor of discussing the disease. The goal is to get you to recognize the patterns of disease and not be bogged down with the intricacies of envelope glycoproteins.

All paramyxoviruses are ss(–)RNA viruses, and so are helical, enveloped, and not cytolytic. All paramyxoviruses induce cell-to-cell fusion, generating **syncytia**—multinucleated giant cells—just like herpesvirus did. But paramyxoviruses do not cause intranuclear inclusions. All paramyxoviruses are spread through **respiratory droplets** and cause subsequent disease. Parainfluenza and RSV cause problems for the lungs, and do not enter the bloodstream. Measles and mumps start in the lungs, then go viremic to infect the tissue they like.

Parainfluenza causes **croup**, a tracheo-laryngo-bronchitis. At first glance, you might see "influenza" and want to associate signs and symptoms of flu to this disease. Do not. Croup does cause "lung disease," a local disease of the site of infection. Viremia does not occur. Croup affects young children, usually aged 4–10, who present with a **seal-like barking cough**. The child is usually not toxic and can be treated in an ambulatory setting. There is usually no fever, myalgias, or malaise. Sometimes, simply going outside into cooler air dissipates the symptoms. Croup is usually discussed relative to epiglottitis because it is "a thing in the throat of young children." That serves only to confuse the two, so we are NOT going to discuss epiglottitis here. Parainfluenza is a **mild lung disease**. It has no treatment, no vaccine, and no sequelae. Parainfluenza is seasonal, occurring more often in the winter.

Respiratory Syncytial Virus, like parainfluenza, is spread by droplets and causes an infection local to the respiratory tract; it does not go viremic. RSV can present with varying intensity, from the common cold through severe pneumonia. Rhinorrhea and a mild temperature elevation is how older children and adults experience the disease. How infants, especially premature neonates, experience the disease is **bronchiolitis** (bronchiole-itis). This inflammation of the small airways produces an effect similar to asthma. Air can get in on inhalation, but has trouble getting out on exhalation, provoking **asthma symptoms** in a child who is **not asthmatic**. They present with cough, wheezing, and even changes on X-ray. Bronchodilators and corticosteroids usually have benefit (treating as if the patient has asthma).

Ribavirin, previously thought to add benefit, is not useful. There is no vaccine and no sequelae. RSV is seasonal, occurring more often in the winter.

The **measles** virus is called rubeola. Measles is **highly infectious** and spreads by respiratory droplets. After replicating in the respiratory tract, it hops onto monocytes and lymphocytes, is circulated first through the lymphatics, then into a cell-associated viremia. The viremia allows the virus access to the conjunctiva, respiratory tract, blood vessels, and CNS. After infection, there is a prodromal state which includes the mouth and eyes—"3 C's and a P." The patient presents with **cough, coryza,** and **conjunctivitis**, which induces **photophobia**. Following that, **Koplik spots** (small white spots on the buccal mucosa) on the inside of the mouth appear, followed finally by a maculopapular rash starting behind the ears, working its way down onto trunk and extremities. The rash is caused by T-cell activation targeting measles in the endothelial cells of blood vessels. A rare complication of measles is **subacute sclerosing panencephalitis (SSPE)**, a rare, late, progressive neurological disease that occurs months to years after the measles, presenting with personality changes, memory changes, spastic jerks, and blindness. You will not see SSPE in the Unites States, and active measles infections are rare, since we have a vaccine against it. The **live attenuated measles virus vaccine** is part of the MMRV.

MMRV vaccine covers measles, mumps, rubella, and varicella. That is how we recommend you learn the names. Be careful, though. A test may attempt to confuse you. Rubeola causes measles, rubella causes German measles. We like to coach the use of the MMRV vaccine as the way to remember that measles is one thing, rubella is the other, and we drop the synonyms from our memory.

Mumps virus follows a similar pathogenesis to measles. The virus is spread by respiratory droplets. After replicating in the respiratory tract, it gets into the blood. The viremia gives mumps access to the cells it likes the most. The only difference is that mumps prefers a different set of tissues than measles did. Mumps like the gonads and the parotid gland. There is a prodromal phase characterized by fever, malaise, and headache followed by **parotitis**. Male adults can get **orchitis** (inflammation of the testes). Orchitis can lead to sterility. The **live attenuated mumps virus vaccine** is part of the MMRV.

Rhabdovirus = Rabies

Rabies virus is a huge **bullet-shaped**, **enveloped** virus. It is **not cytolytic**, but it is fatal. Rabies is transmitted by the bite of a symptomatic, infected animal (bats and racoons in the United States, not dogs). Rabies replicates in the skeletal muscle of the bite site with minimal symptoms (**incubation phase**). The proximity of the bite to the central nervous system and the concentration of virus injected into the wound determines the duration of the incubation phase. Regardless, the incubation phase is long, 30–60 days. After a long incubation phase, the virus infects the peripheral nerve and ascends (**prodrome phase**). The prodrome phase is a vague viral syndrome—fever, malaise, headache, anorexia—and pain at the bite site, which, by a month later, has healed. New pain or itching at the bite site is a sign the virus is spreading. When it reaches the brain, it causes classic symptoms—altered mental status, coma, and eventually death (**neurologic phase**). The neurologic phase also includes **hydrophobia** (fear of water), the most characteristic symptom of rabies, triggered by the pain associated with the patient's attempt to swallow water. During the end of the neurologic phase the virus spreads to glands, including salivary. A bite from the infected animal introduces the virus from the salivary glands into the muscle of a new victim. This happens in animals as well as humans, though humans do not foam at the mouth nor suffer increased aggression the way animals do. Because the incubation period is so long, both **immediate immunoglobulin** (passive immunity) and **repeated post-exposure vaccination** (active immunity) will work—the treatment is immunity, not antivirals.

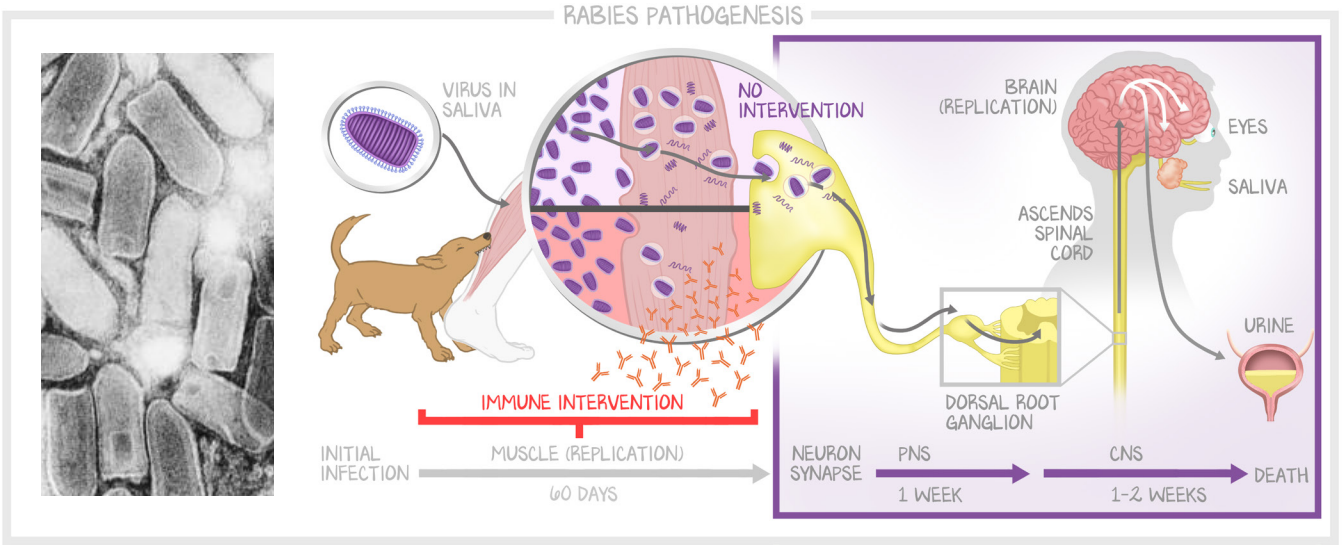

Figure 5.2: Rabies
On the left is a scanning electron micrograph of the bullet-shaped Rhabdovirus. The rest of the illustration reads from left to right. A rabid animal bites a human, injecting virus into the muscle. For 60 days there is opportunity for immune intervention. Either vaccinate to develop innate immunoglobulins or administer preformed antibodies. If immunity is achieved, the virus will not enter the sensory nerve. If immunity is not achieved, the virus will ascend the spinal cord. Once in the brain, it is too late for intervention. Virus will appear in the saliva and the urine as the person dies. Intervention must be made prior to the virus accessing the nerve. Urine and saliva become positive far too late in the disease course.

The virus binds to acetylcholine receptors at nerve endings in muscle and ascends with **retrograde axoplasmic** transport. Once in the spinal cord, the brain rapidly becomes affected. Without treatment, **rabies is always fatal**. By the time laboratory testing becomes positive in the patient who is infected, the virus has already escaped the skeletal muscle into the nerve. The urine and saliva become positive only in the neurologic phase. By the time the disease has progressed that far, the patient is dead. The goal is to have **antibodies present before the virus makes the leap** from skeletal muscle to nerve. If antibodies are present at the end of the incubation phase, as soon as the virus shows itself, it is picked off by antibodies.

The diagnosis can be made by finding **Negri bodies** (eosinophilic cytoplasmic inclusions) in neurons—to get them you must do a brain biopsy, aka autopsy. That's great if we have access to the animal doing the biting and can confirm that .the animal has rabies, but usually the animal is not available. We have ELISA testing that detects the presence of antibody, but antibody is not protective because it is formed late in the disease. By the time the ELISA is positive, it is too late for treatment. We have a means of detecting **RNA in the saliva and urine**, but since the treatment is immunity, waiting to find replicating virus means it is too late to start the treatment. Instead, the decision to vaccinate and give immunoglobulin is dependent on the animal—if the animal behaves rabidly, treat. If not, observe the animal and wait.

Orthomyxovirus = Influenza

Orthomyxovirus is influenza, the virus that causes the flu.

Orthomyxovirus is linear, **segmented**, and **the only negative-sense virus** that can replicate in the nucleus AND the cytoplasm. It is spread by **airborne droplets**. Isolation and masks can be used to reduce transmission. **Influenza A** and **Influenza B** are the flu strains we see in the United States. Flu season is in the late fall to early spring, the cold winter months. Many diseases are said to have "flu-like symptoms." Symptoms of the flu are high fever, chills, myalgia, headache, nausea, and vomiting, followed by respiratory symptoms including a dry cough. The syndrome is usually short-lived, less than 72 hours,

and if there is no preexisting pulmonary disease, is generally well tolerated. It is the robust immune response that causes you to hurt, have fevers, and not want to move—the virus isn't cytolytic but your immune system eliminating the virus is. At one time the killed vaccine was developed in eggs, such that egg allergy was an indication for the intranasal live attenuated vaccine. **Flu vaccinations with killed vaccine are now recommended for everyone and are no longer made in eggs.**

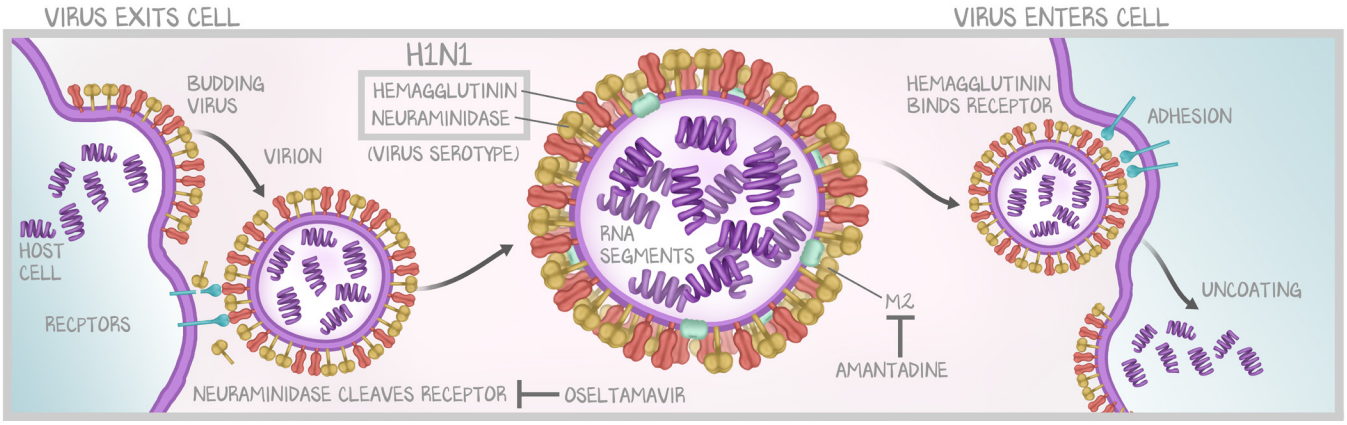

Figure 5.3: Influenza
Orthomyxovirus, the virus that causes influenza, the flu, is a spiral, segmented, ss(–) virus. Its pieces assemble into an enveloped virion which separates from the host cell via neuraminidase. Neuraminidase inhibitors (oseltamivir) are thought to reduce flu symptom duration. The virion binds to the next host cell using hemagglutinin. Orthomyxoviruses are thus categorized by their hemagglutinin and neuraminidase proteins. The envelope protein M2 aids with uncoating. Amantadine, an M2 inhibitor, was thought to help flu symptom duration. It does not.

Influenza is classified by its **H** protein (hemagglutinin) and **N** protein (neuraminidase). For example, the H1 and N1 of "H1N1 influenza" simply define the serotype of those proteins. The H protein is the **attachment** and **fusion** protein that facilitates entry into the cell. Influenza is not cytolytic, and so virus particles need to bud off and leave the host cell. The N protein severs any attachment proteins, and releases the virus particle from the host cell. Neuraminidase inhibitors, **oseltamivir** and zanamivir, are used to reduce flu symptom duration. There are two M proteins. M1 proteins are the **matrix proteins** beneath the membrane and facilitate assembly. M2 is the **membrane protein** that facilitates uncoating. **Amantadine** is an ineffective influenza treatment that targets the M2 protein. Influenza's genome consists of **eight separate** helical nucleocapsid segments, each of which contains the negative-sense RNA, a nucleoprotein, and the RNA polymerase components. This segmentation promotes **genetic diversity** because of **antigenic drift** (point mutations in antigens that change variability slightly) but more so from **antigenic shift** (reassortment of RNA gene segments between two influenza viruses). This is why we need the **flu vaccine annually**. It isn't because we forget what we were vaccinated against, or that influenza isn't immunogenic. The H protein is responsible for the generation of protective antibodies and is quite immunogenic. It's just that there is so much antigen variation that each year a new flu strain appears that our immunoglobulins do not recognize.

Fun Fact: This year's flu vaccine will be based on last year's most prevalent flu types. Because we need time to make the flu vaccine, we can't wait to observe this year's season. The flu vaccine initiative is based on guesswork and on hope that there hasn't been too much genetic variation between years.